thermoscientific

# Comparison of Whole Blood & Precipitated Blood for the Quantitation of Drugs of Abuse Using PaperSpray

Katherine L. Walker, Yu Zhu, and Neloni Wijeratne  Thermo Fisher Scientific, San Jose, CA 95134


## ABSTRACT

**Purpose:** To compare drug of abuse quantitation in untreated whole blood and whole blood that has been precipitated by zinc sulfate in methanol.

**Methods:** Twenty-one drugs of abuse and their corresponding internal standards were prepared in whole blood and methanol. Spiked whole blood was mixed in a 1:3 ratio with a precipitation solution (2:1 methanol:0.2 $ZnSO_4$), centrifuged down, and the supernatant was removed for analysis. The spiked whole blood, precipitated blood, and methanol were deposited onto VeriSpray sample plates, dried, and then analyzed using a Thermo Scientific™ VeriSpray™ PaperSpray ion source coupled to a Thermo Scientific™ TSQ Altis™ MS. The one-minute chronograms were integrated using TraceFinder 5.1 software and calibration curves were generated.

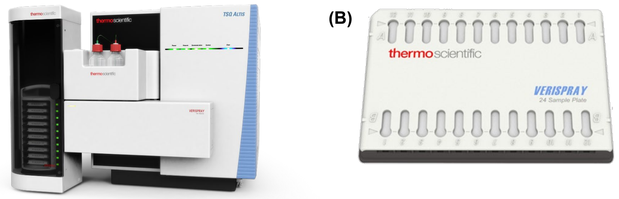
**Results:** For drugs in methanol, all had an LLOQ of 5 ng/mL. In whole blood, the signal of calibrators and the background signal dropped due to matrix effects on ionization. For three benzodiazepines, the signal-to-noise and LLOQ was improved by the precipitation method. These compounds either suffer from decomposition or from protein-binding in whole blood. For the remaining 18 drugs of abuse, precipitation does not improve quantitation.


## INTRODUCTION

PaperSpray-MS is a technique for rapidly quantifying analytes in dried matrix spots such as whole blood. Little or no sample preparation is required, and sample analysis times are 2 minutes or less. The Thermo Scientific™ VeriSpray™ PaperSpray ion source system utilizes PaperSpray technology to make clinical research workflows faster and more efficient by combining ease-of-use and increased automation with the speed that PaperSpray technology provides. The VeriSpray system consists of the VeriSpray ion source and the Thermo Scientific™ VeriSpray™ plate loader (Figure 1A). Each VeriSpray sample plate contains 24 paper strips (12 on each side, A and B, Figure 1B). The plate loader allows a full 10-plate magazine with a total of 240 samples to be run without user intervention.

**Figure 1. (A) VeriSpray ion source and plate loader with loaded magazine mounted to TSQ Altis mass spectrometer (B) VeriSpray sample plate.**

(A) (B)




Since PaperSpray-MS is a direct analysis technique with no chromatographic separation, the sample matrix can decrease the analyte signal and the LLOQ (lower limit of quantitation). Precipitation of proteins from spiked whole blood with zinc sulfate and extraction with methanol can improve analyte signal, especially for compounds that bind to albumin and other proteins. Furthermore, the matrix effects are lessened from the methanolic solution of precipitated blood compared to whole blood.

In this study we use PaperSpray-MS to analyze 21 drugs of abuse in methanol, whole blood, and precipitated blood to determine the utility of protein precipitation.

## MATERIALS AND METHODS

### Sample Preparation

- A mix of 21 drugs in the benzodiazepine, opiate, cocaine, stimulant and sedative classes were spiked into human whole blood or methanol (f.c. 5-400 ng/mL) with corresponding internal standards (IS; f.c. 130 ng/mL).
- Samples were put on a blood shaker for 20-30 minutes.
- The precipitated blood sample was prepared from spiked blood: 100 uL of spiked blood was mixed with 300 uL precipitation solution (2:1 methanol: 0.2M $ZnSO_4$). Vortex and store in fridge for 10 mins. Centrifuge 10 min 12000 rpm. Transfer out 200 uL supernatant.
- For each condition (in methanol, in whole blood, in precipitated blood), 5 replicates of each calibrator level, a matrix blank, and a matrix blank with IS were spotted on VeriSpray sample plates (spotting volume = 8 µL).
- Sample plates were oven-dried at 45 °C for 5 mins and 25 mins for precipitated blood and whole blood, respectively. Samples in methanol dried in 5 mins at r.t.

### PaperSpray Conditions

Rewetting (10 µL) and spraying (100 µL) solvents were both 95:5:0.01 methanol: water: acetic acid. The paper tip to MS inlet distance was set to 6.5 mm.

### Mass Spectrometer Conditions & Data Analysis

The analysis of drugs of abuse was carried out on a TSQ Altis MS connected with the VeriSpray system. Table 1 and 2 show the MS source parameters and optimized MS transitions, respectively. TraceFinder 5.1 Software was used for processing the 1-minute chronograms.

**Table 1. (A) TSQ Altis MS source conditions and (B) time dependent applied spray voltage setting.**

(A)

| Ion Source Parameter | Value |
|---|---|
| Spray Voltage | Time Dependent |
| Ion Transfer Tube Temp | 400 °C |
| Q1 Resolution | 0.7 |
| Q3 Resolution | 1.2 |
| CID Gas | 1.5 mTorr |

(B)

| Time (min) | Voltage (V) |
|---|---|
| 0 | 0 |
| 0.1 | 4000 |
| 0.95 | 0 |

**Table 2. Optimized MS transitions for drugs of abuse on TSQ Altis MS with acquisition time of 1.0 min**

| Compound | Precursor (m/z) | Product (m/z) | Coll. Energy (V) |
|---|---|---|---|
| Norketamine HCl | 224.084 | 124.97 | 24.55 |
| Norketamine HCl | 224.084 | 179.268 | 15.49 |
| Ketamine HCl | 238.099 | 124.97 | 27.87 |
| Ketamine HCl | 238.099 | 207.054 | 14.35 |
| PCP (Phencyclidine) | 244.206 | 86.125 | 12.62 |
| PCP (Phencyclidine) | 244.206 | 91.054 | 31.03 |
| PCP (Phencyclidine) | 244.206 | 159.137 | 13.97 |
| N-ethylpentylone HCl | 250.144 | 174.125 | 30.99 |
| N-ethylpentylone HCl | 250.144 | 189.125 | 23.87 |
| N-ethylpentylone HCl | 250.144 | 202.155 | 18.61 |
| N-ethylpentylone HCl | 250.144 | 205.125 | 13.8 |
| Diphenhydramine HCl | 256.17 | 165.125 | 42.53 |
| Diphenhydramine HCl | 256.17 | 167.125 | 13.26 |
| Nordiazepam | 271.063 | 140 | 27.33 |
| Nordiazepam | 271.063 | 208.083 | 27.33 |
| Diazepam | 285.079 | 153.988 | 26.52 |
| Diazepam | 285.079 | 193.071 | 31.03 |
| Diazepam | 285.079 | 222.125 | 26.15 |
| Diazepam | 285.079 | 257.071 | 21.76 |
| Hydromorphone | 286.144 | 128.071 | 55 |
| Hydromorphone | 286.144 | 157.071 | 38.53 |
| Hydromorphone | 286.144 | 185.071 | 29.56 |
| Morphine | 286.144 | 152.054 | 55 |
| Morphine | 286.144 | 165 | 41.69 |
| Morphine | 286.144 | 181.071 | 35.79 |
| Morphine | 286.144 | 201.012 | 24.92 |
| Norhydrocodone HCl | 286.144 | 171.107 | 36.55 |
| Norhydrocodone HCl | 286.144 | 199.232 | 26.99 |
| Norhydrocodone HCl | 286.144 | 241.107 | 23.7 |
| Benzoylecgonine | 290.139 | 105.125 | 29.14 |
| Benzoylecgonine | 290.139 | 168.196 | 18.73 |
| Hydrocodone | 300.159 | 128.143 | 55 |
| Hydrocodone | 300.159 | 171.125 | 40.17 |
| Hydrocodone | 300.159 | 199.125 | 29.18 |
| Hydrocodone | 300.159 | 243.083 | 22.9 |
| Codeine | 300.159 | 165.071 | 39.54 |
| Codeine | 300.159 | 215.012 | 25.85 |
| Codeine | 300.159 | 225.083 | 27.41 |
| Temazepam | 301.074 | 176.97 | 37.98 |
| Temazepam | 301.074 | 193.054 | 33.85 |
| Temazepam | 301.074 | 239.042 | 46.95 |
| Temazepam | 301.074 | 255.054 | 21.64 |
| Oxymorphone | 302.139 | 198.125 | 41.77 |
| Oxymorphone | 302.139 | 227.083 | 27.75 |
| Cocaine | 304.154 | 82.125 | 29.47 |
| Cocaine | 304.154 | 182.179 | 18.94 |
| Zolpidem | 308.176 | 219.095 | 53.82 |
| Zolpidem | 308.176 | 221.155 | 38.53 |
| Zolpidem | 308.176 | 235.137 | 34.15 |
| Alprazolam | 309.09 | 205 | 40.59 |
| Alprazolam | 309.09 | 274.226 | 24.33 |
| Alprazolam | 309.09 | 281.071 | 25.35 |
| Clonazepam | 316.048 | 190.202 | 42.74 |
| Clonazepam | 316.048 | 214.071 | 36.09 |
| Clonazepam | 316.048 | 269.982 | 24.42 |
| Midazolam | 326.085 | 222.083 | 46.91 |
| Midazolam | 326.085 | 249 | 36.97 |
| Midazolam | 326.085 | 291.083 | 26.19 |
| Zolpidem Phenyl-4-carboxylic acid | 338.15 | 219.137 | 54.24 |
| Zolpidem Phenyl-4-carboxylic acid | 338.15 | 265.083 | 35.71 |
| Zolpidem Phenyl-4-carboxylic acid | 338.15 | 293 | 26.19 |

## RESULTS

Calibration curves for each drug of abuse were generated for analyte in methanol, blood, and precipitated blood. The lower limit of quantification (LLOQ) was set to the lowest calibration standard analyzed that yielded < 20% accuracy and < 15% CV for 5 replicate samples. Example calibration curves of a representative drug in the benzodiazepine, opiate, cocaine, and sedative classes in whole and precipitated blood are shown in Figure 2.

In methanol, the LLOQ was 5 ng/mL, the lowest level calibration standard. In whole blood, the signal of calibrators and the signal of the background decreased compared to results in methanol due to matrix effects. The signal-to-noise was reduced for analytes in blood.

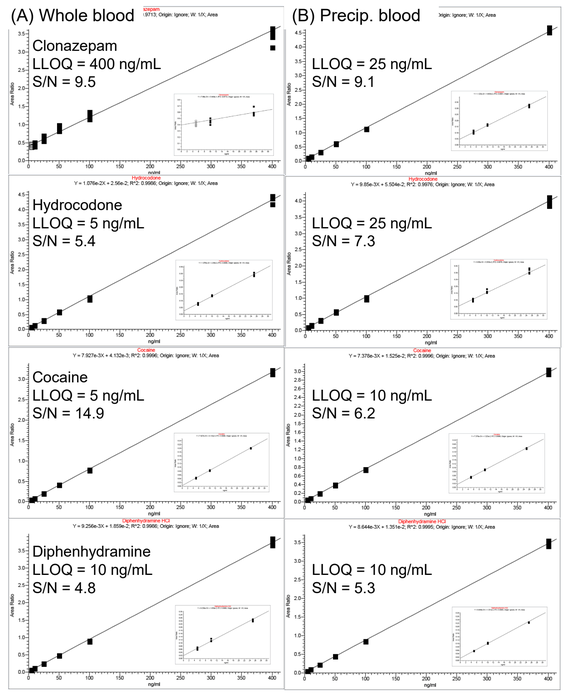
In precipitated blood, the signal of the background was between the values for those in whole blood and methanol. Depending on the analyte, the signal of calibrators either increased or remained the same when compared to the signal in whole blood. For three benzodiazepines—alprazolam, clonazepam, and temazepam—the LLOQ in precipitated blood decreased and the linearity of the calibration curve improved when compared to whole blood (Table 3). For nine analytes—mostly benzodiazepines, stimulants and sedatives—the LLOQ remined the same for whole blood and precipitated blood. For the remaining nine analytes—mostly opiates and cocaine—the LLOQ decreased up to 4-times due to the 4-fold dilution during the preparation of analyte in precipitated blood.

**Table 3. LLOQs for drugs of abuse in methanol (neat), in blood, and in precipitated blood.**

| Compound | LLOQ Neat (ng/mL) | LLOQ in Whole Blood (ng/mL) | LLOQ in Precip Blood (ng/mL) |
|---|---|---|---|
| Alprazolam | 5 | 10 | 5 |
| Benzoylecgonine | 5 | 10 | 25 |
| Clonazepam | 5 | 400 | 25 |
| Cocaine | 5 | 5 | 10 |
| Codeine | 5 | 25 | 100 |
| Diazepam | 5 | 25 | 25 |
| Diphenhydramine | 5 | 10 | 10 |
| Hydrocodone | 5 | 5 | 25 |
| Hydromorphone | 5 | 50 | 50 |
| Ketamine | 5 | 5 | 25 |
| Midazolam | 5 | 5 | 5 |
| Morphine | 5 | 25 | 50 |
| N-ethylpentylone | 5 | 25 | 25 |
| Nordiazepam | 5 | 5 | 5 |
| Norhydrocodone | 5 | 10 | 50 |
| Norketamine | 5 | 10 | 25 |
| Oxymorphone | 5 | 25 | 50 |
| PCP | 5 | 5 | 5 |
| Temazepam | 5 | 400 | 25 |
| Zolpidem Phenyl-4-carboxylic acid | 5 | 5 | 5 |
| Zolpidem | 5 | 5 | 5 |

## RESULTS CONT.

**Figure 2. Calibration curves of clonazepam, hydrocodone, cocaine, and diphenhydramine in (A) whole blood and (B) precipitated blood. Inset: zoom from 1-30 ng/mL.**




## CONCLUSIONS

- PaperSpray is a sensitive, direct technique for the analysis of drugs of abuse in whole blood. Sample preparation is not necessary for a majority of analytes.
- For benzodiazepines, precipitation with zinc sulfate and methanol is a simple, rapid clean-up method to improve the LLOQ. In particular, clonazepam and temazepam improved significantly in precipitated blood because they bind strongly to albumin.
- For difficult compounds, especially known protein-binders, precipitation with zinc sulfate is a simple, rapid clean-up method that should be considered when optimizing sample preparation.

## REFERENCES


1. Espy, R.D. et al. Paper spray and extraction spray mass spectrometry for the direct and simultaneous quantification of eight drugs of abuse in whole blood. *Anal Chem.* **2014**, 86, 15, 7712-7718.
2. Chin, P.K.L et al. Adult age and *ex vivo* protein binding of lorazepam, oxazepam and temazepam in healthy subjects. *Br J Clin Pharmacol.* **2011**, 72, 6, 985-989.
3. Pacifici, G.M. et al. Plasma protein binding of clonazepam in hepatic and renal insufficiency and after hemodialysis. *Ther Drug Monit.* **1987**, 9, 4, 369-373.


## TRADEMARKS/LICENSING

© 2020 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others.

PO65747-EN 0422S

ThermoFisher SCIENTIFIC